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[RAPID COMMUNICATION]

Immunohistochemical Study of Ontogeny of Pituitary Prolactin and Growth Hormone Cells in *Xenopus laevis*

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ABSTRACT—The ontogeny of prolactin (PRL) and growth hormone (GH) cells in the pars distalis of *Xenopus laevis* was examined immunohistochemically using anti-bullfrog PRL serum and anti-bullfrog GH serum. Immunoreactive PRL and GH cells first appeared simultaneously at Nieuwkoop and Faber (NF) stage 42, in the anterodorsal region and in the central region of the pars distalis, respectively. Immunoreactive PRL cells increased moderately as metamorphosis progressed. They were distributed mainly in the anterior portion of the pars distalis. Immunoreactive GH cells showed a marked increase in number at NF stages 50–52 and NF stages 62–64 and a slight decrease at the end of metamorphosis. Throughout late premetamorphosis, prometamorphosis and climax, the GH cell number always exceeded the prolactin cell number. GH cells were situated in the posterior portion of the pars distalis. Examination of consecutive sections stained alternately with anti-PRL and anti-GH did not reveal colocalization of PRL and GH at any stage of development.

INTRODUCTION

Prolactin (PRL) and growth hormone (GH) belong to a family of hormones that are functionally and structurally related [8]. In amphibians, PRL stimulates growth of larval organs such as gills and tail and GH stimulates somatic growth [10]. Amino acid sequences of bullfrog PRL and GH have been determined by direct protein sequencing [12, 24] or deduced from their cDNAs [20, 21]. The two proteins exhibit a considerable sequence homology. Recently, co-localization of PRL and GH in the pituitary of bullfrog larvae at early developmental stages has been reported [9, 13]. Ontogenic differentiation of pituitary GH and/or PRL cells in several species of amphibians has been studied immunohistochemically using antisera against GH and/or PRL of mammalian origin [3, 4, 7, 14, 18, 29]. Recently, however, antisera against PRL [25] and GH [11] of amphibian origin have also become available. The antiserum against bullfrog PRL stained PRL cells in adult *Rana ridibunda, Pleurodeles waltlii, Ambystoma mexicanum, Xenopus laevis, Bufo vulgaris* and *Triturus cristatus* [1, 2, 16]. The antiserum against bullfrog GH has been applied to *Rana ridibunda* [28], *Bufo vulgaris, Bufo japonicus* and *Xenopus laevis* [17]. However, ontogenic studies of amphibian PRL and GH cells using these antisera have been limited to only two species, namely, *Rana catesbeiana* [13] and *Rana dalmatina* [5]. The present study was carried out to study the development of GH and PRL cells in *Xenopus* larvae, paying particular attention to coexistence of PRL and GH within the same cell.

MATERIALS AND METHODS

Animals Fertilized eggs of *Xenopus laevis* were obtained by

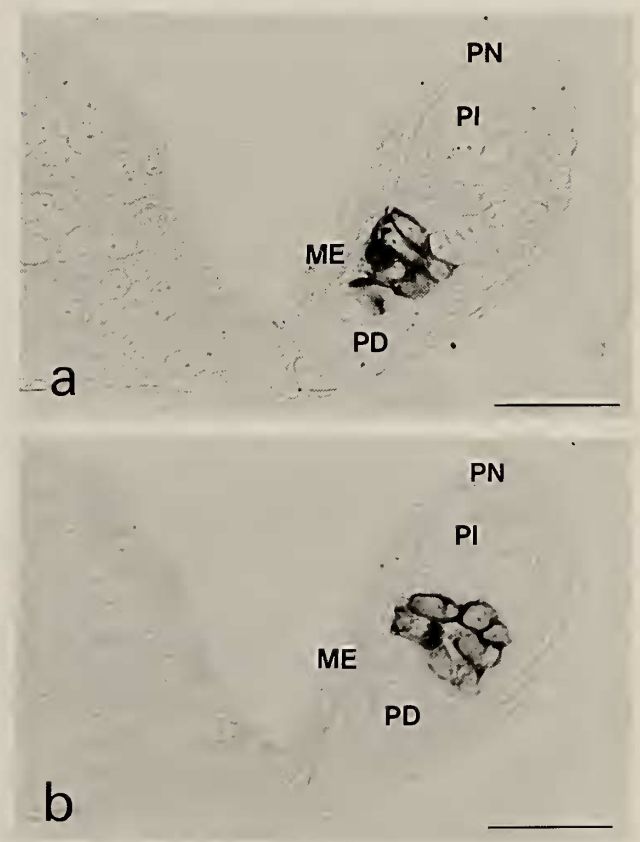

FIG. 1. The consecutive mid-sagittal sections of the pituitary gland of larval *Xenopus* (NF stages 42) stained with anti-bullfrog RPL serum (a) and anti-bullfrog GH serum (b). ME, median eminence; PD, pars distalis; PI, pars intermedia; PN, pars nervosa. Bar, 20 μm.

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injection of 200–400 IU human chorionic gonadotropin (Teikokuzoki Co, Tokyo) into mature male and female animals. The hatched embryos were reared under laboratory conditions until use. The larvae were staged according to Nieuwkoop and Faber (NF) [15]. In addition to larvae, several juvenile animlas (one month after metamorphosis) were used.

Immunohistochemistry The whole brains were fixed for 24 hr in Bouin's solution. After dehydration and embedding in paraplast, serial sagittal sections (5 μm) were cut and mounted on gelatin-coated slides. The deparaffinized sections were incubated in a solution of 0.3% H_2O_2 in methanol for 30 min. After rinsing 3 times with phosphate-buffered saline (PBS) (pH 7.2), the slides were treated with normal swine serum (1:20) for 1 hr. After washing with PBS, the sections were immunostained by the peroxidase anti-peroxidase (PAP) method [19]. Sections were incubated sequentially with the following: rabbit anti-bullfrog PRL serum (1:2000) [25] or anti-bullfrog GH serum (1:2000) [12], swine anti-rabbit IgG (1:20) (Dako Japan, Kyoto) for 2 hr and rabbit PAP complex (1:50) (Dako Japan, Kyoto) for 1.5 hr. The section were stained with 10 mg of 3,3′-diaminobenzidine tetrahydrochloride and 0.005% H_2O_2 in 100 ml of Tris-HCl buffer (pH 7.6), rinsed with distiled water, stained with 1% methyl green, dehydrated in 100% isopropanol and xylol and mounted in Bioleit. The number of

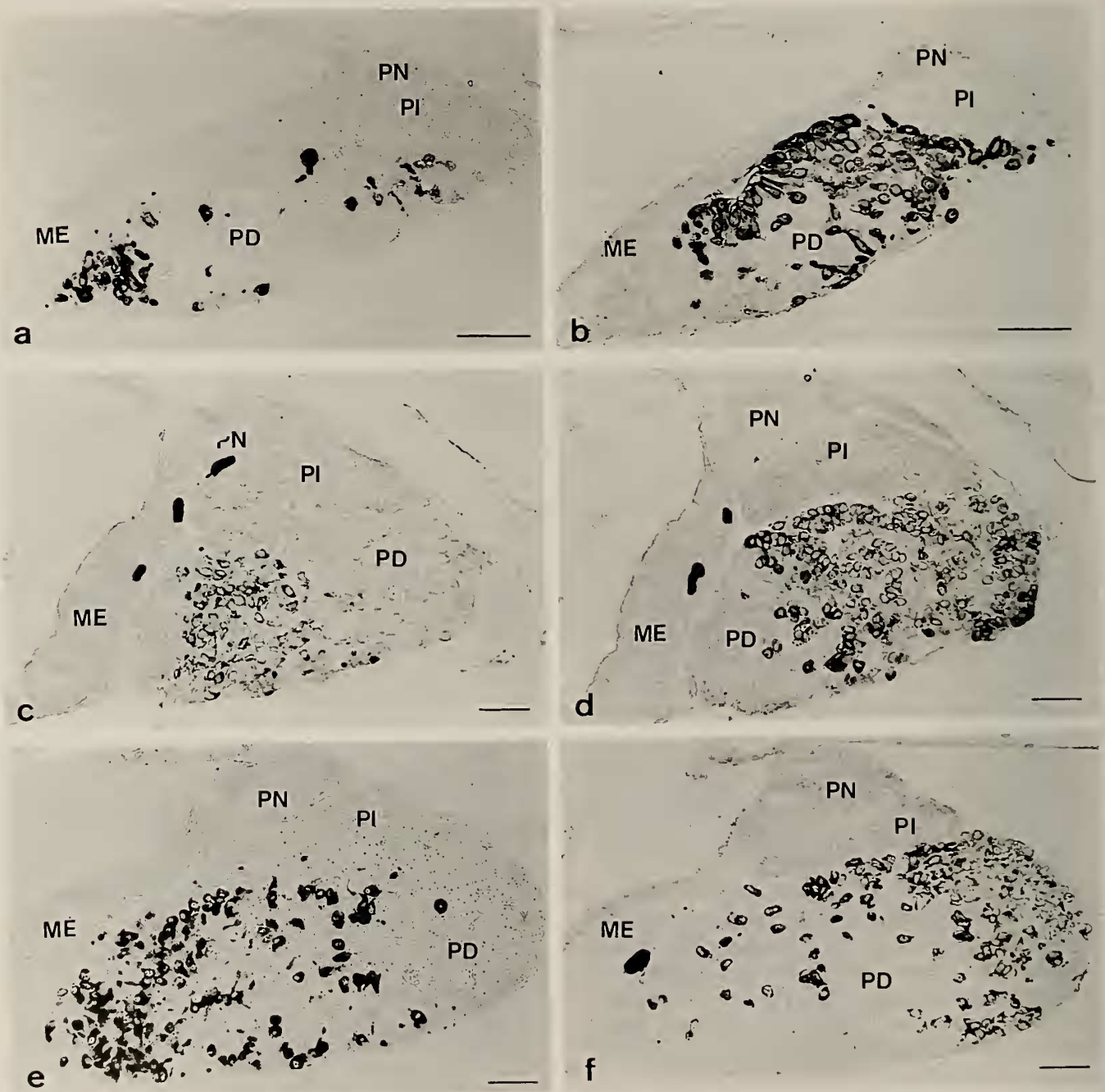

FIG. 2. Mid-sagittal sections of pituitary gland showing localization of immunoreactive PRL (a, c and e) and GH (b, d and f) cells in NF stages 55 (a and b), NF stages 62 (c and d) and juvenile (e and f) *Xenopus laevis*. ME, median eminence; PD, pars distalis; PI, pars intermedia; PN, pars nervosa. Bar, 50 μm.

immunoreactive cells with a visible nucleus in mid-sagittal section was counted to use as an index of the cell population [24]. The values from five specimens of each group were expressed as mean ± standard error of the mean (SEM). Student's *t* test was used for statistical analysis. Control sections were incubated with normal rabbit serum or antisera preadsorbed with an excess of the corresponding antigen instead of the specific antiserum.

RESULTS

The intensity of the immunoreaction was considerable with the antisera against bullfrog PRL and bullfrog GH. No reaction was observed when sections were incubated with normal rabbit serum instead of the antiserm against bullfrog PRL or bullfrog GH. Immunostaining was completely abolished when sections were incubated with the primary antisera preadsorbed with corresponding antigens (data not shown).

At NF stage 42 (embryonic nonfeeding stage), immunoreactive PRL cells first appeared in the anterodorsal region of the pars distalis (Fig. 1a). Almost simultaneously, GH-immunoreactive cells appeared more caudally than PRL cells (Fig. 1b). There was an apparent segregation of PRL and GH groups. Comparison of two consecutive sections stained with anti-bullfrog PRL and anti-bullfrog GH, respectively, did not show colocalization of PRL and GH (Fig. 1, a and b).

At the subsequent premetamorphic stages (NF stages 43-54), the increase in PRL cell population was not so marked (Fig. 3). During prometamorphosis (NF stages 55–61) and climax (NF stages 62-64), PRL cells increased in number moderately. They were located mainly in the anterior portion of the pars distalis (Fig. 2, a and c). On the other hand, a marked increase of GH cell number was observed during the late premetamorphic period (NF stages 50–52). Thereafter, the population of GH cells became 2–3 times larger than that of PRL cells. Again, the increase of GH cell number occurred during early climax (NF stages 62–64). At the end of metamorphosis, a slight but significant decrease of the number of GH cells was observed (Fig. 3). Immunoreactive GH cells were abundant in the caudal portion of the pars distalis (Fig. 2, b and d). In juveniles, localization of PRL and GH cells was fundamentally the same as that in larvae. PRL cells were situated mainly in the rostral portion and GH cells were in the caudal portion of the pituitary gland (Fig. 2, c and e). The presence of PRL cells with occasional long processes was noted. No apparent coexistence of PRL and GH was observed in the pituitary gland of larvae at advanced metamorphic stages or of juveniles.

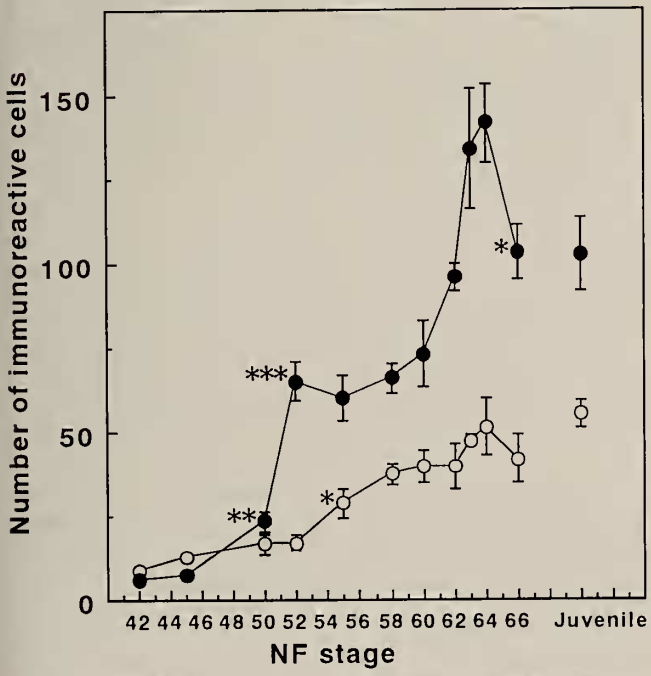

FIG. 3. Population of PRL (clear circles) and GH (solid circles) cells detected in mid-sagittal sections of *Xenopus* larvae at various developmental stages and of juveniles. The values are expressed as mean of 5 determinations ± S.E.M. Significant differences at $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ versus preceding stage (Student's *t* test).

DISCUSSION

Moriceau-Hay *et al.* [14] have studied the development of PRL and GH cells in *Xenopus* tadpoles using antisera against bovine PRL and GH. According to them, PRL cells first appeared at stage 42. This is consistent with the present result. However, they were able to first recognize immunoreactive GH cells only at stage 44, whereas we detected them at stage 42. This discrepancy may be due to the difference in sensitivity of the antisera used in these two experiments. Moriceau-Hay *et al.* [14] stated that the cross-reactivity of the anti-bovine GH serum they used was quite low. This is often the case when antisera against GH of mammalian origin are used for the detection of GH cells in amphibian hypophyses [5].

In this study, we observed that PRL cells were less abundant than GH cells throughout prometamorphosis and climax. This persisted even one month after metamorphosis. However, with the same antisera as those used in this study, we have confirmed that the population of PRL cells exceeds that of GH cells when the toads become adult [17]. It has been reported that, in *Rana esculenta,* an anti-ovine GH serum stains both PRL and GH cells [23]. Immunological studies by Hayashida [6] also demonstrated cross-reactivity between frog PRL and antiserum against primate GH. In the present study, we used antisera against RPL and GH of amphibian origin. The specificity of these antisera has been confirmed by radioimmunoassay [1, 11, 25] and immunoblotting [13, 17, 28]. Using these anti-PRL and anti-GH sera, coexistence of PRL and GH in secretory granules within the same cells in the pituitary gland of embryonic bullfrogs has been demonstrated [9, 13]. In this study, however, colocalization of PRL and GH in *Xenopus* pituitary cells was not observed. Failure to demonstrate the

coexistence of PRL and GH within the same cell of *Rana dalmatia* pituitaries has also been reported by Guastalla *et al.* [5].

Recently, we have isolated two molecular forms of both PRL and GH [26, 27] from the *Xenopus* pituitary gland. These two forms of PRL and GH showed considerable cross-reactivity with the anti-bullfrog PRL and anti-bullfrog GH, respectively. Production of specific antiserum against each hormonal molecule is under way. If these specific antisera become available and are applied to immunohistochemistry and radioimmunoassay, more precise information about PRL and GH cell function in *Xenopus* larvae could be obtained.

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